

NICKEL ACCUMULATION IN SERPENTINE ARTHROPODS FROM THE RED HILLS, CALIFORNIA

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Abstract.—Serpentine soils are characterized by high levels of heavy metals (e.g., Ni, Fe, Cr), and low levels of important plant nutrients (e.g., P, Ca, N). Due to these inhospitable edaphic conditions, serpentine soils are typically home to a very specialized flora. Although much is known about the serpentine flora, almost no research has investigated the arthropods of serpentine areas. In this study, we sampled the arthropods associated with *Streptanthus polygaloides* (Gray), a Ni hyperaccumulator, and the arthropod community of the surrounding serpentine area in the Red Hills of California. Arthropods were then analyzed for Ni content to investigate Ni transport within the serpentine ecosystem. Arthropods associated with *S. polygaloides* contained significantly higher concentration of Ni than those collected in the surrounding community. One insect associated with *S. polygaloides*, *Melanotrichus boydi* Schwartz and Wall (Hemiptera: Miridae), accumulated 770 μg Ni/g.

Key Words.—Insecta hyperaccumulation, serpentine arthropods.

Whereas some heavy metals are essential nutrients and are crucial to the survival of most organisms, in excessive doses they also can be toxic. At many sites heavy metal concentrations in the environment are artificially elevated due to anthropogenic influences such as mining and metal smelting. Because it is possible in some cases to know when human-caused metal contamination began (Brooks 1998), many authors have been able to conduct research on rates of adaptation of organisms to metal contamination (e.g., Wu et al. 1975, Posthuma 1990). This research is not only interesting from a microevolutionary standpoint but also has revealed physiological and genetic mechanisms by which organisms adapt to high concentrations of metal in the environment. However, the relatively recent history of anthropogenic metal contamination at mines and smelters precludes studying the effects of heavy metals on these human-impacted ecosystems over longer time spans.

Serpentine soils provide an opportunity to study long-term effects of heavy metals on ecosystems. Distributed around the globe, serpentine soils are high in heavy metals such as Ni, Fe, and Cr but are low in important plant nutrients like Ca and P (Brooks 1987). Within North America, there are extensive areas of serpentine soils in California and Oregon (Kruckeberg 1984). Whereas Coleman (1967) suggested that these serpentinized areas of California and Oregon have been exposed since the Tertiary, a more recent hypothesis by Raven and Axelrod (1978) suggests a much younger origin for these sites (3 to 24 million years ago). In either case, plant and animal species in these areas have had millions of years in which to adapt to the unique edaphic conditions provided by serpentine soil.

The serpentine flora of California has been the subject of botanical research since the late 1800's (Kruckeberg 1984). Much of this research has focused on

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the adaptations of plants to the unique edaphic conditions of serpentine soils (Brooks 1987). Of particular interest has been the accumulation of Ni by plants. Serpentine plant species commonly accumulate higher levels of Ni than nonserpentine species (Brooks 1987). Moreover, some species of serpentine plants, termed "hyperaccumulators," contain over 1000 $\mu\text{g Ni/g}$ (Brooks et al. 1977). These concentrations of Ni have been found to defend plants against many types of herbivores (see reviews by Boyd and Martens 1998, Boyd 1998). The high Ni concentration in Ni hyperaccumulators provides a unique environment to which herbivores must adapt in order to utilize these plants as a food source.

Many authors have hypothesized that there should be a unique fauna associated with the serpentine flora (Proctor and Woodell 1975, Kruckeberg 1984, Brooks 1987), but little research has specifically addressed this idea. The purpose of this study was to assess interspecific differences in the Ni concentration of arthropods associated with a serpentine community with an emphasis on insects associated with metal hyperaccumulating plants.

MATERIALS AND METHODS

Study Site.—This study was conducted in the Red Hills Management Area in Tuolumne County, California. The entire management area is underlain with serpentine soils (Franklin et al. 1997). Much of the community-level sampling in this study took place in a large (ca. 2000 m^2) area along Red Hills Road in the Red Hills Management Area. The vegetation was dominated by *Ceanothus cuneatus* (Hook.) Nutt., *Clarkia biloba* (Durand) Nels & Macbr., *Calycadenia multiglandulosa* ssp. *bicolor* (Greene) Keck, and *Streptanthus polygaloides* Grey. Species-specific sampling took place throughout the Red Hills Management Area, but was concentrated at the location described above.

Community-level Sampling.—We sampled arthropods within the general serpentine community of the Red Hills Management Area via both pitfall-trapping and black-lighting. In June 1996 and 1997, 21 pitfall traps were placed at the study site. Traps were arranged in an approximately 30 m by 70 m grid. Traps were placed approximately 10 m apart. A trap consisted of a 14 cm diameter by 12 cm deep plastic cup set into the ground so that the lip of the cup was level with the soil surface. Cups contained approximately 100 ml of a 50:50 mixture of ethylene glycol and water. Cups were covered by a 22 cm diameter plastic plate to reduce evaporation of preservative. A 2–3 cm space was left between the cover and the soil surface to allow arthropods to easily enter traps.

For black lighting, on three occasions over a one week period in June of 1996 five black-lights were spaced at approximately 50 m intervals throughout a site (ca. 2000 m^2) in the Red Hills Recreational Area. Lights were turned on around 9 pm and allowed to shine against a 1 m^2 white sheet. We sampled lights at 10 pm, 11 pm, and 12 am. During a collecting bout, arthropods were arbitrarily collected for approximately 5–10 minutes at each light.

Species-Specific Sampling: Streptanthus Polygaloides.—Accompanying the broad community-level sampling of the Red Hills, we also specifically sampled the arthropods associated with *S. polygaloides*, the only known Ni hyperaccumulator in the Red Hills. *Streptanthus polygaloides* is endemic to serpentine barrens of the foothills of the western part of the Sierra Nevada from Fresno County, California north to Butte County, California (Kruckeberg 1984). Containing an

average of 9750 $\mu\text{g Ni/g}$ (Reeves et al. 1981, Kruckeberg & Reeves 1995), *S. polygaloides* is an annual that often grows in relatively dense stands.

Streptanthus polygaloides was sampled via both sweep-netting and visual inspection. Sampling took place during June of 1996, 1997, and 1998 while the plants were in flower. Dense pure stands of *S. polygaloides* were targeted for sweep-netting in order to reduce inadvertent sampling of other plant species.

Apis mellifera L. and *Bombus vandykkee* (Frison) were used to compare Ni concentrations found within members of the same species that occur both on and off serpentine soils. Both species were collected from *S. polygaloides* in the Red Hills and from *Heteromeles arbutifolia* (Lindley) Roemer, a shrub growing on a nonserpentine site > 15 km from the Red Hills, for comparison.

Elemental Analysis.—Specimens were sorted according to morphotype and representatives of each morphotype were pinned and labeled for later identification to the lowest taxonomic level that could be readily attained. Individual specimens were air-dried for at least 72 h at 67° C and weighed. Individuals of the same morphotype weighing less than 50 mg were combined in order to create samples of at least that mass for analysis. Specimens were then analyzed for Ni concentration as described below. In order to sample variation in Ni values adequately, we analyzed at least three samples of each morphotype to generate means (\pm SD). Many of our morphotypes could not be analyzed for Ni concentration, due to their low mass. Morphotypes with insufficient biomass to create three samples for analysis are not included in the data presented here.

Nickel concentration was determined with an atomic absorption spectrophotometer (Instrumentation Laboratory, IL 251). Samples were digested in borosilicate glass test tubes using 3–5 ml of concentrated nitric acid at 110° C for 6–8 h, after which time most of the liquid had evaporated. The residue was then redissolved in 3–5 ml of 1 M hydrochloric acid at 110° C for 2–4 h. The solutions were then diluted with distilled water to a volume of 10 or 25 ml, depending on the original mass of the dried sample. Reagent blanks were made and processed with every batch of samples in order to correct for any contamination generated by the technique. All metal values are reported as $\mu\text{g metal/g}$ on a dry weight basis.

Specimens containing unusually high levels of Ni (> 300 $\mu\text{g Ni/g}$) were also analyzed for Cr in order to rule out contamination by soil. Chromium levels are several orders of magnitude higher in serpentine soils than in the plants growing on serpentine soils. Unusually high levels of Ni accompanied by high levels of Cr indicate the potential of soil contamination (Brooks 1987). Concentrations of Cr were determined via inductively coupled argon plasma spectrophotometry (Jarrell-Ash, ICAP 9000).

Data Analysis.—Nickel concentrations in arthropod tissues were analyzed by one-way analysis of variance (ANOVA) in order to determine if association with *S. polygaloides* influenced specimen Ni concentrations. Nickel concentrations were log-transformed in order to satisfy the assumptions of ANOVA (Zar 1984). Log-transformed Ni concentrations of hemipteran herbivores collected on *S. polygaloides* were also analyzed via one-way ANOVA. In this case, post-hoc mean separations were performed using Fisher's Protected Least Significant Difference (PLSD) test (SAS Institute 1998) in order to compare Ni concentrations between pairs of hemipteran species. Vouchers of analyzed specimens were deposited in

Table 1. Nickel concentration (mean \pm SD) of insect species or morphospecies associated with *Streptanthus polygaloides* in the Red Hill Recreational Area, California.

Order Family	Morphospecies or species	Mean \pm SD	n
Coleoptera			
Bruchidae	<i>Acanthoscelides seminulum</i> Horn	55 \pm 96	3
Melyridae	Mel-1	129 \pm 18	3
Diptera			
Otitidae	Oti-1	58 \pm 8	3
Hemiptera			
Miridae	<i>Melanotrichus boydi</i> Schwartz & Wall	777 \pm 162	8
	<i>Lygus hesperus</i> Knight	131 \pm 126	3
Pentatomidae	<i>Thyanta pallidovirens</i> (Stål)	40 \pm 28	3
Rhopalidae	<i>Liorhyssus hyalinus</i> (Fabricus)	48 \pm 50	4
Hymenoptera			
Apidae	<i>Apis mellifera</i> L.	43 \pm 24	10
	<i>Bombus vandykei</i> (Frisson)	38 \pm 34	10
Halictidae	<i>Dialictus</i> sp.	51 \pm 27	3
Lepidoptera			
Lycaenidae	<i>Everes amyntula</i> (Boisduval)	36 \pm 34	3
Overall Mean		128 \pm 218	

the University of Connecticut insect collection with voucher labels with the prefix, “wall-boyd-CA” followed by the morphospecies names listed in Tables 1 and 2.

RESULTS

We collected a total of 110 morphotypes of arthropods. Almost half of these arthropods (50) were collected in association with *S. polygaloides*. The remaining arthropods (60) were collected by black-lighting and pitfall-trapping. Of all the morphotypes collected, only 33 were collected in great enough number to allow us to analyze three replicates. Eleven of the 33 analyzed morphotypes were collected in association with *S. polygaloides*. The arthropods analyzed contained an average of 65 \pm 132 μ g Ni/g. However, one plant bug associated with *S. polygaloides*, *Melanotrichus boydi* Schwartz and Wall, contained an average of 777 \pm 162 μ g Ni/g (Table 1) (also see Schwartz and Wall 2001). *Melanotrichus boydi* contained almost no Cr (1 \pm 2 μ g Cr/g, *n* = 3). If samples of *M. boydi* are excluded, the average Ni content of the arthropods sampled decreases to 43 \pm 34 μ g Ni/g.

Nickel content of arthropods associated with *S. polygaloides* (Table 1) was significantly higher than the Ni content of arthropods collected via black-lighting and pitfall-trapping (Table 2, ANOVA: *F* = 11.45; *df* = 1, 31; *P* = 0.002). Even when *M. boydi* is excluded from this analysis, there is still significantly more Ni in arthropods associated with *S. polygaloides* than in arthropods collected via black-lighting and pitfall-trapping (ANOVA: *F* = 8.69; *df* = 1, 30; *P* = 0.006).

Melanotrichus boydi contained more Ni than other hemipteran herbivores found feeding on *S. polygaloides*. Other than *M. boydi*, three other hemipteran herbivores were collected from *S. polygaloides* in great enough numbers to analyze: *Lygus hesperus* Knight (Heteroptera: Miridae), *Thyanta pallidovirens* (Stål) (Heteroptera: Pentatomidae), and *Liorhyssus hyalinus* (F.) (Heterptera: Rhopalidae).

Table 2. Nickel concentrations (mean \pm SD) of insect species or morphotypes collected via pitfall traps and black lights in the Red Hills Recreational Area, California.

Order Family	Morphospecies or species	Mean \pm SD	n
Coleoptera			
Elateridae	Ela-1	41 \pm 69	3
Eucnemidae	Euc-1	23 \pm 15	3
	Euc-2	19 \pm 15	3
Tenebrionidae	<i>Eleodes</i> sp.	75 \pm 71	3
Lepidoptera			
Geometridae	Geo-1	7 \pm 7	3
	Geo-2	8 \pm 14	3
	Geo-3	13 \pm 23	3
	Geo-4	62 \pm 80	3
	Geo-5	13 \pm 16	3
	Geo-6	11 \pm 13	3
	Geo-7	13 \pm 12	3
Noctuidae	Noc-1	40 \pm 59	3
	Noc-2	32 \pm 24	3
Pyrilidae	Pyr-1	12 \pm 21	3
	Pyr-2	44 \pm 69	3
	Pyr-3	12 \pm 22	3
Neuroptera			
Corydalidae	Cor-1	32 \pm 21	3
Myrmeleontidae	Myr-1	13 \pm 7	3
Orthoptera			
Acrididae	<i>Malanopus</i> sp.	40 \pm 15	5
Gryllacrididae	<i>Ceuthophilus</i> sp.	51 \pm 28	3
Gryllidae	<i>Gryllus assimilis</i> (Fabricius)	133 \pm 77	3
Tettigoniidae	<i>Arethea</i> sp.	55 \pm 28	4
Overall Mean		34 \pm 29	

All of these insects were observed feeding on the young stems and leaves of *S. polygaloides* in the field. Also, the presence of nymphs of *L. hesperus*, *M. boydi*, and *T. pallidovirens* on *S. polygaloides* supports the hypothesis that *S. polygaloides* can be a host for these herbivores. These herbivores varied significantly in Ni content (ANOVA: $F = 8.36$; $df = 3, 15$; $P = 0.002$). Although all these herbivores appeared to be feeding on *S. polygaloides*, *M. boydi* contained significantly more Ni than the other three species of hemipterans (Fisher's PLSD test: $P = 0.001, 0.008$, and 0.002 for pairwise comparisons of *M. boydi* with *L. hesperus*, *T. pallidovirens*, and *L. hyalinus* respectively).

Apis mellifera collected while visiting flowers of *S. polygaloides* contained significantly higher levels of Ni ($46 \pm 8 \mu\text{g Ni/g}$, $n = 10$) than those collected from *Heteromeles arbutifolia* ($16 \pm 8 \mu\text{g Ni/g}$, $n = 10$) in a non-serpentine environment (ANOVA: $F = 7.88$; $df = 1, 18$; $P = 0.012$). Like *A. mellifera*, *Bombus vandykei* collected from *S. polygaloides* contained significantly more Ni ($38 \pm 15 \mu\text{g Ni/g}$, $n = 5$) than those collected from *H. arbutifolia* ($12 \pm 14 \mu\text{g Ni/g}$, $n = 5$); (ANOVA: $F = 8.60$; $df = 1, 8$; $P = 0.019$).

DISCUSSION

Historically, little attention has been paid to accumulation of Ni by arthropods in terrestrial environments (see Hopkin 1989). This may be due to the typically

low levels of Ni found in arthropods from metal-contaminated sites (e.g., Helioväara and Vaisanen 1990, Helioväara et al. 1990, Bagatto and Shorthouse 1996). On the other hand, a study of arthropods collected from serpentine sites in Zimbabwe reported very high levels of Ni accumulation (Wild 1975). Termites collected from these sites contained 5000 $\mu\text{g Ni/g}$ and 1500 $\mu\text{g Cr/g}$ (Wild 1975). Wild (1975) hypothesized that these high levels of Ni and Cr were the result of accumulation from ingested plant material. In plant material, however, high levels of Cr are typical of soil contamination on specimens (Brooks 1987). As with Wild's (1975) work with termites from the serpentine exposures of Zimbabwe, there is the potential that residual plant material in the gut and/or dust on specimens may have artificially elevated Ni concentrations in *Melanotrichus boydi*. Soil/dust contamination is unlikely unless Cr concentrations in a sample exceed 100 $\mu\text{g Cr/g}$ (Brooks 1987). Levels of Cr in *M. boydi*, averaging only 1 $\mu\text{g Cr/g}$, are extremely low and thus soil/dust contamination seems unlikely to be the source of Ni in the specimens analyzed.

There are several alternatives that may explain the disparity in Ni content found amongst herbivores of *Streptanthus polygaloides*. In one scenario, metal content varies between tissues and organs of the plant and the observed differences in herbivore metal content simply reflect the differences in the plant tissue or organ type upon which was fed. In this study, the herbivores with the three lowest Ni values, *Thyanta pallidovirens*, *Liorhyssus hyalinus*, and *Acanthoscelides semimulum*, were collected on the developing fruits of *S. polygaloides*. Indeed, the developing fruits (1100–5230 $\mu\text{g Ni/g}$) of *S. polygaloides* are known to contain less Ni than leaves (3300–14,800 $\mu\text{g Ni/g}$) or flowers (2860–16,400 $\mu\text{g Ni/g}$) (Reeves et al. 1981). On the other hand, both mirid species, *M. boydi* and *Lygus hesperus*, feed on the same plant organs, young developing leaves and flowers. Thus it is difficult to explain the disparity in metal content of the two mirids, as being due to differences in organ metal content. These differences could, however, be due to variation in metal content between tissues. Boyd and Martens (1999) found that aphids were able to feed upon *S. polygaloides* without accumulating significant amounts of Ni. The implication being that the aphids contained little Ni because the phloem sap contains little Ni. Unlike aphids, no mirids are known to tap into sieve elements and feed directly on phloem sap (Wheeler 2001). While feeding on phloem sap is unlikely to explain the low metal content in *L. hesperus* relative to *M. boydi*, the two species could target tissues of differing metal content within *S. polygaloides*. Unfortunately, nothing is known about the distribution of metal between tissues in *S. polygaloides*.

The alternative scenario is that these species feed on similar tissues but vary in their ability to accumulate and/or excrete metal. If the insects are unable to avoid metal, then excretion and storage-detoxification are the two major strategies for terrestrial invertebrates dealing with toxic levels of heavy metal (Dallinger 1993). To effectively distinguish which strategy is employed by which species would require laboratory-based studies with artificial diets as opposed to our own field-based studies. It is interesting to note, however, that previous work has shown *M. boydi* to be a specialist on *S. polygaloides* (Wall 1999) and *L. hesperus* is widely known to be extremely polyphagous (Schwartz & Footit 1998). Thus differences in strategy may represent different "trade-offs" by specialist versus polyphagous herbivores (Futuyma & Moreno 1988).

We found that arthropods associated with a Ni hyperaccumulator, *S. polygaloides*, contained more Ni than arthropods collected from the general serpentine community. Arthropods collected from *S. polygaloides* also contained more Ni than arthropods reported in the literature that were collected from sites contaminated with Ni (e.g., Helioväara and Vaisanen 1990, Helioväara et al. 1990). The higher levels of Ni found in arthropods associated with *S. polygaloides* probably reflect the degree to which hyperaccumulators accumulate Ni, as well as the bio-availability of that Ni. Although plants from Ni-contaminated sites typically contain relatively low levels of Ni (e.g., Koricheva and Haukioja 1995), *S. polygaloides* contains an average of 9750 $\mu\text{g Ni/g}$ (Reeves et al. 1981). Often, Ni contamination at polluted sites is the result of aerial deposition and therefore is only available in an inorganic form (Berthelsen et al. 1995). Within hyperaccumulators, Ni appears to be complexed with an organic acid (Lee et al. 1978) and is potentially more available to arthropods.

The variation in metal content that we observed within insect orders highlights the need to separate taxa to finest taxonomic level that material allows. In their study on metal content of insects associated with Scottish serpentine soils, Davison et al. (1999) pool their specimens together at the ordinal level for metal analysis. This does not allow exploration of intraordinal variation in metal content, which our study suggests, can be significant. For instance, mean values of Ni within the Hemiptera ranged from $40 \pm 28 \mu\text{g Ni/g}$ in *T. pallidovirens* to $777 \pm 162 \mu\text{g Ni/g}$ in *M. boydi*. From this perspective, our own study would have been more informative had we the biomass to analyze different body parts in order to isolate Ni localization within the body.

The variation of Ni content that we observed in this study offers a unique system upon which comparative studies can be built. For instance, both an endemic specialist (i.e., *M. boydi*) and cosmopolitan generalists (e.g., *T. pallidovirens*, and *L. hesperus*) feed on *S. polygaloides*. These two groups differ significantly in metal content (Table 1), yet the function of elevated Ni content in *M. boydi* and the physiological mechanisms by which it sequesters Ni remain unclear. Research that contrasts the physiological mechanisms of metal tolerance in *M. boydi* with those of generalist herbivores could address both of these issues.

ACKNOWLEDGMENT

We would like to thank T. Henry, P. Naskrecki, M. Schwartz, and D. Rider for assisting in identification of specimens. Furthermore, we thank M. Davis, A. Teem and E. Watkins for assistance in the field and laboratory.

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Received 28 December 2000; Accepted 20 June 2002.